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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 5/06, 5/08, A61K 37/64 — A1

(11) International Publication Number: WO 92/04370

(43) International Publication Date: 19 March 1992 (19.03.92)

(21) International Application Number: PCT/US91/05691

(22) International Filing Date: 9 August 1991 (09.08.91)

(30) Priority data:
574,634 29 August 1990 (29.08.90) US

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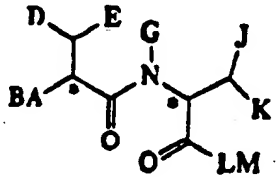
(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KP, KR, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MODIFIED DI- AND TRIPEPTIDYL IMMUNOSUPPRESSIVE COMPOUNDS



(I)

(57) Abstract

A series of linear, modified di- and tripeptides are claimed, which have an affinity for FK-506 binding protein (FKBP) and are immunosuppressive agents. These compounds, which are characterized by high lipophilicity and the absence of charged groups, can be depicted by general formula (I). These compounds are useful as T-cell specific immunosuppressive agents for the treatment of graft rejecti n, graft versus host disease, and a wide variety of autoimmune diseases in an individual.

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MODIFIED DI- AND TRIPEPTIDYL IMMUNOSUPPRESSIVE COMPOUNDS

Background of the Invention

Post operative graft rejection is a major complication affecting the success of bone marrow and organ transplantations. However, through the use of immunosuppressive drug therapy, graft rejection in organ transplantation can be significantly reduced.

A wide variety of diseases can be characterized as "autoimmune diseases". Such diseases are similar to graft rejection, except that the rejection is of self tissue. Immunosuppressive therapy can also be of use in prevention or treatment of this inappropriate self rejection.

One widely accepted immunosuppressant for the prevention of graft rejection is cyclosporin A (CsA). It is a natural product of fungal metabolism and has been demonstrated to have potent immunosuppressive activity in clinical organ transplantations. Calne, R.Y. et al., Br. Med. J. 282:934-936 (1981); White, D.J.C. Drugs 24:322-334 (1982). Although CsA is widely used in immunosuppressant therapy, its usage (particularly in high dosage) is often accompanied by side effects which include nephrotoxicity, hepatotoxicity and other central nervous system disorders.

The following diseases have been treated with cyclosporin A with positive results, confirming the importance of the autoimmune component in these diseases and their effective treatment with a compound which works by selective suppression of T-cell immune function.

1) Ophthalmology: Uveitis, Behcet's disease and Grave's ophthalmopathy.

Weetman, A.P. et al., Lancet 486-489 (1982). Grave's opthalmopathy.

Nussenblatt, R.B. et al., Lancet 235-238 (1983). Uveitis.

French-Constant, C. et al., Lancet 454 (1983). Behcet's disease.

Sanders, M. et al., Lancet 454-455 (1983). Behcet's disease.

Note: Cyclosporin A is currently approved in Japan for the treatment of Behcet's disease, the first autoimmune disease indication for this compound.

2) Dermatology: Various autoimmune skin diseases including psoriasis.

Zabel, P. et al., Lancet 343 (1984). Acute dermatomyositis.

van Joost, T. et al., Arch. Dermatol. 123:166-167 (1987). Atopic skin disease.

Appleboom, T. et al., Amer. J. Med. 82:866-867 (1987). Scleroderma.

Logan, R.A. and R.D.R. Camo, J. Roy. Soc. Med. 81:417-418 (1988). Eczema.

Griffiths, C.E.M. et al., Brit. Med. J. 293:731-732 (1986). Psoriasis.

Ellis, C.N. et al., J. Amer. Med. Assoc. 256:3110-3116 (1986). Psoriasis.

3) Hematology: Various diseases including anemia.

Toetterman, T.H. et al., Lancet 693 (1984). Pure red cell aplasia (PRCA).

Stryckmans, P.A. et al., New Engl. J. Med. 310:655-656 (1984). Aplastic anemia.

Gluckman, E. et al., Bone Marrow Transplant 3 Suppl. 1, 241 (1988). Aplastic anemia.

4) Gastroenterology/Hepatology: Primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease and other gastrointestinal autoimmune diseases.

Wiesner, R.H. et al., Hepatology 7:1025, Abst. #9, (1987). Primary biliary cirrhosis.

Hyams, J.S. et al., Gastroenterology 93:890-893 (1987). Autoimmune hepatitis.

Allison, M.C. et al., Lancet 902-903 (1984). Crohn's disease.

Brynskov, J. et al., Gastroenterology 92:1330 (1987). Crohn's disease.

Porro, G.B. et al., Ital. J. Gastroenterol. 19:40-41 (1987). Ulcerative colitis.

5) Neurology: Amyotrophic lateral sclerosis (ALS, "L u Gehrig's disease"), myasthenia gravis and multiple sclerosis.

Appel, S.H. et al., Arch. Neurol. 45:381-386 (1988). ALS.

Tindall, R.S.A. et al., New Engl. J. Med. 316:719-724 (1987). Myasthenia gravis.

Dommasch, D. et al., Neurology 38 Suppl. 2, 28-29 (1988). Multiple sclerosis.

6) Nephrotic Syndrome: Nephrotic syndrome, membrano-proliferative glomerulonephritis (MPGN) and related diseases.

Watzon, A.R. et al., Clin. Nephr 1. 25:273-274 (1986). Nephrotic syndrome.

Tejani, A. et al., Kidney Int. 33:729-734 (1988). Nephrotic syndrome.

Meyrier, A. et al., Transplant Proc. 20, Suppl. 4 (Book III), 259-261 (1988). Nephrotic syndrome.

LaGrue, G. et al., Nephron. 44:382-382 (1986). MPGN.

7) Rheumatoid Arthritis (RA)

Harper, J.I. et al., Lancet 981-982 (1984). RA.

Van Rijthoven, A.W. et al., Ann. Rheum. Dis. 45:726-731 (1986). RA.

Dougados, M. et al., Ann. Rheum. Dis. 47:127-133 (1988). RA.

8) Insulin-Dependent Diabetes Mellitus (IDDM)

Stiller, C.R. et al., Science 223:1362-1367 (1984). IDDM.

Assan, R. et al., Lancet 67-71 (1985). IDDM.

Bougneres, P.F. et al., New Engl. J. Med. 318:663-670 (1988). IDDM.

Diabetes 37:1574-1582 (1988). IDDM.

Many veterinary diseases are also characterized as autoimmune diseases. Autoimmune diseases such as those listed above have been observed in mammals. Papa, F. O. et al., Equine Vt. J. 22:145-146 (1990), infertility of autoimmune origin in the stallion; Gorman, N.T. and L. L. Werner, Brit. Vet. J. 142:403-410, 491-497 and 498-505

(1986), immune mediated diseases of cats and dogs; George, L.W. and S.L. White, Vet. Clin. North Amer. 6:203-213 (1984), autoimmune skin diseases in large mammals; Bennett, D., In. Pract. 6:74-86 (1984), autoimmune diseases in dogs; Halliwell, R. E., J. Amer. Vet. Assoc. 181:1088-1096 (1982), autoimmune diseases in domesticated animals.

The mechanism by which CsA causes immunosuppression has been established. In vitro, CsA inhibits the releas of lymphokines, such as interleukin 2 (IL-2) (Bunjes, D. et al., Eur. J. Immunol. 11:657-661 (1981)), and prevents clonal expansion of helper and cytotoxic T cells (Larsson, E. J. Immunol. 124:2828-2833 (1980)). CsA has been shown to bind to the cytosolic protein, cyclophilin, and to inhibit the associated peptidyl-prolyl cis-trans isomerase (PPIase) activity of that protein. Fischer, G. et al., Nature 337:476-478 (1989); Takahashi, N. et al., Nature 337:473-475 (1989).

Recently, a second natural product isolated from Streptomyces, referred to as FK-506, has been demonstrated to be a potent immunosuppressive agent. Tanaka, H. et al., J. Am. Chem. Soc. 109:5031-5033 (1987). Like cyclosporin A, FK-506 inhibits IL-2 production, the mixed lymphocyte culture response, and the generation of cytotoxic T-cells in vitro; however, FK-506 is effective in these assays at concentrations 100 times lower than cyclosporin A. Kino, T. et al., J. Antibiot. 15:1256-1265 (1987). FK-506 is structurally distinct from CsA and binds to a different target protein called FK-506 binding protein (FKBP). Therefore, FKBP may offer an alternative target for immunosuppressive agents.

Summary of the Invention

This invention relates to a novel class of compounds having an affinity for the FK-506 binding protein, as well as to a method of suppressing an immune response in an individual by administering to the individual a quantity of at least one of the compounds sufficient to produce the desired effect (i.e., immune suppression). The compounds are linear, modified di- or tripeptides. As described herein, compounds of the present invention inhibit the peptidyl-prolyl cis-trans isomerase acitivity of the FKBP. Further, the compounds of the present invention can lead to inhibition of T cell activation. The compounds of this invention can be used as immunosuppressive drugs to prevent or reduce graft rejection in bone marrow and organ transplantation and in the treatment of autoimmune disease.

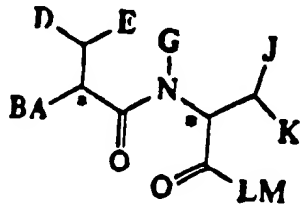
Brief Description of the Figure

The Figure shows the structures of eight compounds of this invention.

Detailed Description of the Invention

This invention relates to a novel class of compounds which have an affinity for FK-506 binding protein (FKBP) and are immunosuppressive agents. Some of these compounds inhibit the peptidyl-prolyl cis-trans isomerase activity of FKBP, and inhibition of the PPIase activity by the compounds appears to be associated with binding of FKBP. Compounds of this invention, however, may have an affinity for other FK-506 binding proteins or other

peptidyl-prolyl cis-trans isomerases, including cyclophilin and as yet unidentified proteins or complexes, and may be immunosuppressive by means of inhibition of the actions of such species. The FKBP-like proteins or complexes can be identified by their affinity for FK-506 or its immunosuppressive analogs. Therefore, reference herein to FK-506 binding protein or FKBP is taken to indicate the FK-506 binding protein or FKBP-like proteins or complexes, identifiable by their affinity for FK-506 or its immunosuppressive analogs. This invention further relates to a method of suppressing (i.e., reducing or preventing) an immune response in an individual, such as a human or other mammal. The compounds are modified di- or tripeptides and have a linear backbone. These compounds, which can be used as immunosuppressive agents, include those represented by the general formula, given below,



In the general formula:

A is NH, O, S or CH.

If A is NH, O, or S, B is PCO- or POCO-, where P is a C1-C6 straight or branched alkyl or alkenyl group,

a C5-C6 cycloalkyl or cycloalkenyl, or a methyl substituted with a C5-C6 cycloalkyl, C5-C6 cycloalkenyl, phenyl, 1-naphthyl, 2-naphthyl, 9-fluorenyl, or 1-adamantyl.

If A is CH, then B is connected via a trans double bond and is a C2-C4 straight or branched alkyl or alkenyl group, or is a methyl or ethyl substituted with either a C5-C6 cyclic alkyl group or Ar, where Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, phenyl and phenyl having one to three substituents which are independently selected from the group consisting of: hydroxyl, halo, nitro, CF_3, C1-C4 straight or branched alkyl or alkenyl, O-(C1-C4) straight or branched alkyl or alkenyl, and Ar, where Ar is selected from the group consisting of: 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, phenyl and phenyl having one to three substituents which are independently selected from the group consisting of: hydroxyl, halo, nitro, CF_3, C1-C4 straight or branched alkyl or alkenyl, O-(C1-C4) straight or branched alkyl or alkenyl; wherein no more than two Ar groups may be linked together.

D is hydrogen; C1-C4 straight or branched alkyl or alkenyl; hydroxy; tert-butyloxy; benzyloxy; 4-benzyloxyphenyl; cyclohexyl; $-(CH_2)_n-CO_2-Q$, where n = 0 or 1 and Q is methyl, ethyl, i-propyl, t-butyl, benzyl, 1-napthyl, 2-napthyl, or cyclohexyl; or Ar, where Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, phenyl and phenyl having one to three substituents which are independently selected

from the group consisting of: hydroxyl, halo, nitro, CF_3, C1-C4 straight or branched alkyl or alkenyl, O-(C1-C4) straight or branched alkyl or alkenyl, and Ar, where Ar is selected from the group consisting of: 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, phenyl and phenyl having one to three substituents which are independently selected from the group consisting of: hydroxyl, halo, nitro, CF_3, C1-C4 straight or branched alkyl or alkenyl, O-(C1-C4) straight or branched alkyl or alkenyl; wherein no more than two Ar groups may be linked together;

E and K are independently hydrogen or methyl.

G is either methyl or ethyl; J is hydrogen, C1-C6 straight or branched alkyl or alkenyl, C5-C6 cycloalkyl or cycloalkenyl, sulfhydryl, hydroxy, phenyl, 3-indolyl, or benzyl; wherein G and J may be connected by a bond to form a cycle of 5 or 6 members.

L is O or is an α-amino acid residue attached via the α-nitrogen, and selected from the group consisting of: alanine, 2-aminobutyric acid, valine, norvaline, leucine, norleucine, isoleucine, phenylalanine, cyclohexylalanine, tryptophan, 1-naphthylalanine, 2-naphthylalanine, threonine (side chain benzyl or tert-butyl ether), glutamic acid (side chain benzyl or tert-butyl ester), methionine, or serine (side chain benzyl or tert-butyl ether).

If L is O, then M is C1-C6 straight or branched alkyl or alkenyl, or $-(CH_2)_n$-Ar, where n = 1-6 and Ar is selected from the group consisting of: 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, phenyl and

phenyl having one to three substituents which are independently selected from the group consisting of: hydroxyl, halo, nitro, CF_3, C1-C4 straight or branched alkyl or alkenyl, O-(C1-C4) straight or branched alkyl or alkenyl, and Ar, where Ar is selected from the group consisting of: 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, phenyl and phenyl having one to three substituents which are independently selected from the group consisting of: hydroxyl, halo, nitro, CF_3, C1-C4 straight or branched alkyl or alkenyl, O-(C1-C4) straight or branched alkyl or alkenyl; wherein no more than two Ar groups may be linked together.

If L is an amino acid, then M is O-(C1-C4) straight or branched alkyl, O-benzyl, NH-phenyl, or NH-4-nitrophenyl and is attached to the amino acid carbonyl.

The stereochemistry at all positions may be (R) or (S). Preferably the stereochemistry is (S) at L if L is an α-amino acid, and at those positions marked with asterisks. However, when J is sulfhydryl, the preferred stereochemistry of the asterisked position immediately adjacent to the nitrogen shown in the general formula is (R).

The structures of eight compounds of the present invention are given in the Figure. These compounds and other compounds of the general formula, with substituents A through M as described above, are linear, modified di- or tripeptides.

The modified peptides of the present invention contain a linear backbone, unlike the macrocyclic antibiotic FK-506 or the cyclic peptide cyclosporin A.

Although the compounds of the present invention may contain cyclic groups, the substituents at the ends of the molecules, specified by B and M, are linked only to the adjacent elements at A and L, respectively, and not to each other.

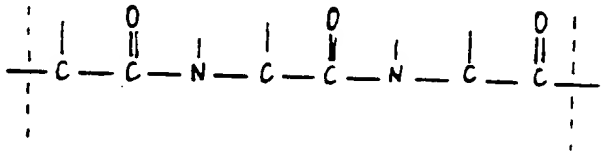
A "modified peptide" of the present invention is a compound with a peptide backbone, in which the amino and/or carboxy termini are altered, and optionally there is at least one internal modification. The amino and/or carboxy termini can be altered so that they have substituents, or the N-terminal amino group may be replaced. Optionally any or all of the backbone amide groups, which are analogous to peptide bonds, may be modified by substitution. The backbone amide group shown in the general formula is typically modified by substitution at G, and optionally, the amino acid side chains may be modified by addition, deletion, or substitution to form side chains not present in proteogenic amino acids.

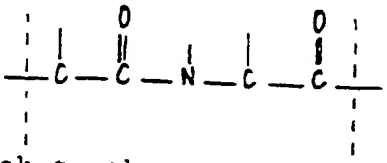
Many types of modification are possible. In terms of the final modified structure, the N-terminal amino group of the peptide can be altered by substitution, as for example at B, or by replacement, as in cases where A is O, S, or CH. The carboxyl group at the C-terminus may also be modified, as occurs with variation at M, for example.

Internal modifications are optional, and compounds with one or more internal modifications are included in the designation "modified peptides". For example, certain substitutions at D, E, G (when G and J are connected by a bond to form a cycle), J and K can lead to

alterations of the groups or side chains attached to the α-carbons (asterisked positions in the general formula). Where L is an α amino acid, its side chain will vary according to the residue selected or by modification of a side chain. The class of compounds defined as "modified peptides" is not meant to be restricted to the specified variants of the general formula. However, compounds which contain charged groups (e.g., amines or carboxylic acids or their salts) are excluded from the class of modified peptides as defined here.

As mentioned above, the linear, modified peptides of this invention are structurally related to short peptides of two or three amino acids. Thus, they can be further defined by a characteristic length, wherein the backbone of the structure is similar in length to that of a peptide of two or three amino acids. Representative peptide backbone structures are shown below.





Referring back to the structures of the compounds shown in the Figure, three amino acid units may be identified in compound 1, while only two amino acid units occur in compound 4. Two of the amino acid units can be identified by the central or α-carbons, which are asterisked in the

general formula. The third amino acid unit is defined by L, when L is an α-amino acid. The elements at B (including P), and at M correspond to protecting groups.

Bulky, hydrophobic groups are preferred at P, which is a substituent of B. Bulky, hydrophobic substituents are also preferred at D. If L is an amino acid, a residue which has a hydrophobic side chain, or one that is modified to be hydrophobic, is preferred. These compounds preferably contain one imino acid residue.

The compounds of this invention have an affinity for FK-506 binding protein, which is found in the cytosol of lymphocytes, particularly T lymphocytes. FKBP, like cyclophilin, is a peptidyl-prolyl cis-trans isomerase; these two proteins comprise the only known enzymes with this activity. FK-506 and CsA act as specific inhibitors of the peptidyl-prolyl cis-trans isomerase activity of their respective binding proteins, with inhibition constants consistent with the observed concentrations necessary to produce cellular immunosuppression (Harding, M.W. et al., Nature 341:758-760 (1989); Siekierka, J.J. et al., Nature 341:755-757 (1989)). Furthermore, recent reports indicate that numerous analogs of FK-506 show a high degree of correlation of these activities (N. Sigal, Merck, Sharp & Dohme, lecture presented at Dana Farber Cancer Center, 1990). These data indicate that compounds which act as inhibitors of the peptidyl-prolyl cis-trans isomerase activity of FKBP (or of cyclophilin) can be expected to act as T-cell specific immunosuppressants.

One particular FK-506 binding protein has been identified by Harding, M.W. et al., Nature 341:

758-760 (1989) and can be us d as a standard by which to evaluate the binding affinity (K_D) of the compounds for FKBP. As noted above, compounds of this invention, however, may have an affinity for other FK-506 binding proteins or other peptidyl-prolyl cis-trans isomerases, including cyclophilin and as yet unidentified proteins or complexes, and may be immunosuppressive by means of inhibition of the actions of such species. The values for K_i of several sample compounds of the present invention and the binding affinities (K_D) of these compounds for FKBP are reported in the Table below.

Table of Compounds

Compound	K_i (μM)	K_D (μM
Fmoc-alanyl-pipecolyl-phenylalanyl-4-nitroanilide (1)	4	0.15
Fmoc-(tert-butyl)-threonyl-prolyl-phenylalanyl-4-nitroanilide (2)	1.6	1.0
Boc-valyl-prolyl-phenylalanyl-4-nitroanilide (3)	14	>10
Fmoc-cyclohexylalanyl-pipecolic acid benzyl ester (4)	7	0.3
Boc-alanyl-prolyl-leucine benzyl ester (5)	200	ND
Fmoc-(tert-butyl)-glutamyl-prolyl-phenylalanyl-4-nitroanilide (6)	1.5	0.1
Boc-alanyl-proline benzyl ester (7)	ND	ND
Boc-alanyl-prolyl-alanine benzyl ester (8)	>250	ND
Boc-alanyl-prolyl-phenylalanine benzyl ester (9)	>500	ND
Boc-alanyl-prolyl-(tert-butyl)glutamic acid methyl ester (10)	500	ND
Boc-alanyl-prolyl-phenylalanine methyl ester (11)	>15	ND
Boc-alanyl-prolyl-phenylalanyl-4-nitroanilide (12)	ND	ND
Boc-leucyl-prolyl-phenylalanyl-4-nitroanilide (13)	ND	ND
Boc-(benzyl)-tyrosyl-proline benzyl ester (14)	1.5	ND
Boc-(benzyl)-tyrosyl-proline tert-butyl ester (15)	50	ND
Fmoc-alanyl-pipecolic acid benzyl ester (16)	5	ND

Human FK-506 binding protein can be obtained as described by Harding, M.W. et al., Nature 341: 758-760 (1989). Values for the apparent K_D can be determined from a competitive LH-20 binding assay performed as described by Harding, M.W. et al., Nature 341:758-760 (1989), using 32-[1-^{14}C]-benzoyl FK-506, or as described by Siekierka, J.J. et al., Nature 341:755-757 (1989) using [^{3}H] dihydro-FK-506. The K_D values reported in Table 1 were obtained using the latter method, where the ability of an unlabeled compound to compete with the binding of [^{3}H] dihydro-FK-506 to FK-506 binding protein was measured.

The inhibition of the PPIase (rotamase) enzyme activity of FKBP by a compound can also be measured as described either by Harding, M.W. et al., Nature 341:758-760 (1989) or Siekierka, J.J. et al., Nature 341:755-757 (1989). The cis-trans isomerization of the alanine-proline peptide bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe4-nitroanilide, is monitored spectrophotometrically in a coupled assay with chymotrypsin, which releases 4-nitroanilide from the trans form of the substrate. Fischer, G. et al., Nature 337: 476-478 (1989). The inhibitory effect of the addition of different concentrations of inhibitor on the extent of the reaction is determined, and analysis of the change in the first order rate constant as a function of inhibitor concentration yields an estimate of the apparent K_i (Table).

Compounds 1, 2, 3, 4, and 6 resemble FK-506 in their affinity for FKBP, and compounds 1, 2, 3, 4, 6. 8, 9, 14,

15, and 16, like FK-506, have been shown to inhibit th peptidyl-prolyl cis-trans isomerase (PPIase) activity of FKBP. Cyclosporin A also has an affinity for cyclophilin and inhibits the associated PPIase activity. For both FK-506 (Fischer, G. et al., Nature 337: 476-478 (1989); Takahashi, N. et al., Nature 337: 473-475 (1989)), and cyclosporin A (Harding, M.W. et al., Nature, 341: 758-760 (1989); Siekierka, J.J. et al., Nature 341:755-757 (1989)), the values determined for the dissociation constants from their respective binding proteins and for the K_i of inhibition of the associated PPIase activities are consistent with the concentrations at which each compound is effective in assays of in vitro cellular immune response, in which T cell activation and proliferation is monitored, Kino, T. et al., J. Antibiot. 15: 1256-1265 (1987). Thus, linear, modified peptide compounds of the present invention can be expected to possess potent in vitro and in vivo immunosuppressive activity. Thus, such compounds can be used as immunosuppressants for prophylaxis of organ rejection or treatment of chronic graft rejection, and for the treatment of autoimmune diseases.

The immunosuppressive compounds of this invention can be periodically administered to a patient undergoing bone marrow or organ transplantation or for another reason for which it is desirable to substantially reduce or suppress a patient's immune response, such as in various autoimmune diseases. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian autoimmune diseases.

Due to their affinity for FKBP, and their inhibition of the PPIase activity of FKBP, the novel compounds of the present invention may possess activity in suppression of antigen-stimulated growth and clonal expansion of T-cells, especially those T-cells characterized as "helper" T-cells. This activity is useful in the primary prevention of organ transplant rejection, in the rescue of transplanted organs during a rejection episode, and in the treatment of any of several autoimmune diseases known to be associated with inappropriate autoimmune responses. These autoimmune diseases include: uveitis, Behcet's disease, Graves ophthalmopathy, psoriasis, acute dermatomyositis, atopic skin disease, scleroderma, eczema, pure red cell aplasia, aplastic anemia, primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease, amyotrophic lateral sclerosis, myasthenia gravis, multiple sclerosis, nephrotic syndrome, membranoproliferative glomerulonephritis, rheumatoid arthritis and insulin-dependent diabetes mellitus. In all of the above-listed autoimmune diseases, treatment is effective to reduce the symptoms and slow progression of the disease. In the case of insulin-dependent diabetes mellitus, treatment as described below is most effective when instituted before the complete cessation of natural insulin production and transition to complete dependence on external insulin.

For these purposes the compounds of the present invention may be administered by a variety of routes (e.g., orally, parenterally, by inhalation spray, topically, nasally, buccally, rectally, vaginally or via an implanted reservoir) in dosage formulations containing

pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intrasternal and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed, including synthetic mono- or di-glycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, as do natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant such as Ph. Helv or similar alcohol.

The compounds may be administered orally, in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include

lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including autoimmune diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be

formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the opthalmic uses, the compounds may be formulated in an ointment such as petroleum.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of 0.01 to 100 mg/kg per day of the active ingredient compound are useful in the treatment of the above conditions. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a veriety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination and the severity of the particular disease being treated.

The compound can also be administered in combination with a steroid, such as methyl prednisalone acetate, for additional immuno-suppressive effect. The steroid is administered orally, intravenously, rectally, topically or by inhalation. Dosages (based upon methyl prednisalone acetate) of 0.1-5 mg/kg/day may be employed. An initial loading dose of 100-500 mg may be employed. Steroid doses may be decreased with time from the higher toward the lower doses as the clinical situation indicates.

The compounds can be administered with other immunosuppressant drugs, such as rapamycin, azathioprine, 15-deoxyspergualin, cyclosporin, FK-506 or combinations of these, to increase the immunosuppressive effect. Administration of cyclosporin and FK-506 together should be avoided due to contraindications reported resulting from coadministration of these immunosuppressants. The dosage level of other immunosuppressant drugs will depend upon the factors previously stated and the immunosuppressive effectiveness of the drug combination.

OKT3, which is a murine monoclonal antibody to CD3 surface antigen of human T lymphocytes, can also be coadministered intravenously with compounds of the present invention for rescue and reversal of acute allograft rejections, particularly in renal transplantations.

The invention will be further illustrated by way of the following examples, which are not intended to be limiting in any way.

EXAMPLES

General

In these experiments, silica gel flash chromatography is carried out as follows: a solution of one part of the compound to be purified is applied as a solution in a suitable solvent, such as dichloromethane or the eluant, to a column composed of 50 to 100 parts of 230-400 mesh E. Merck silica gel, prepacked in the appropriate eluant under 5-10 psi pressure. Additional eluent is forced through the column by application of 5-10 psi pressure, and the effluent is collected in appropriately sized fractions. A 2 μL sample of each fraction is then assayed for the presence and purity of the desired compound by application to a 0.25 mm thick E. Merck $60F_{254}$ silica gel plate and elution with the column eluent. Detection of the compound is carried out by exposure to UV light or by treating the plate with an appropriate staining agent, for example a 10% solution of phosphomolybdic acid in ethanol, and subsequent heating.

Analytical reverse phase HPLC is carried out by the following methods:

Method A:

Column = Waters MicroBondapak C18, 10μM silica, 3.9 mm ID, 30 cm L.

Moble Phases: A = 0.1% H_3PO_4 in H_2O

B = 0.1% H_3PO_4 in acetonitrile

Gradient: T = 0 minutes, A(95%), B(5%)

T = 15 minutes, A(0%), B(100%)

T = 16.5 minutes, A(0%), B(100%)

Flow - 2 mL/minute, Temperature - 23°, Detection by UV absorbance at 214 nm.

Method B:

Column - Waters MicroBondapak C18, 5μM silica, 3.9 mm ID, 15 cm L.

Moble Phase: A - 0.1% H_3PO_4 in H_2O

B - 0.1% H_3PO_4 in acetonitrile

Gradient: T - 0 minutes, A(95%), B(5%)

T - 20 minutes, A(0%), B(100%)

T - 24 minutes, A(0%), B(100%)

Flow - 1.5 mL/minute, Temperature - 23°, Detection by UV absorbance at 214 nm.

Methods for the synthesis of the five of the compounds illustrated in the Figure are described below.

Example 1

Synthesis of Fmoc-alanyl-pipecolyl-phenylalanyl-4-nitroanilide (1)

1. Fmoc-alanyl-pipecolic acid (17)

A suspension of 189 mg (1.50 mmol) of (S)-pipecolic acid in 10 mL of dichloromethane was treated with 0.21 mL (1.7 mmol) of chlorotrimethylsilane and stirred for 1.5 h. The resulting solution was cooled in an ice/water bath and treated sequentially with 520 mg (1.58 mmol) of Fmoc-alanine acid chloride and 742 μL (3.2 mmol) of diisopropylethylamine. The mixture was stirred for 16 h under a nitrogen atmosphere, warming slowly to ambient

temperature. The mixture was poured into 3 volumes of ether and washed twice with 10% aqueous potassium bisulfate and then with water. The organic layer was then treated with saturated sodium bicarbonate solution. An oily yellow third layer was formed, which was collected along with the aqueous layer. The ethereal layer was extracted twice more with aqueous sodium bicarbonate, each time collecting the third layer. The combined aqueous and oily yellow layers were treated with 6N HCl to bring the pH to 1 and extracted three times with ether. These organic extracts were washed with water and saturated sodium bicarbonate solution, then dried over magnesium sulfate. The mixture was filtered and concentrated in vacuo to yield 0.66 g of 17 as a yellow, foamy solid which was used for subsequent reactions without purification.

2. Fmoc-alanyl-pipecolyl-phenylalanyl-4-nitroanilide (1)

A suspension of 460 mg of compound 17,328 mg (1.15 mmol) of (S)-phenylalanyl-4-nitroanilide, and 155 mg (1.15 mmol) of 1-hydroxybenzotriazole monohydrate in 20 mL of dichloromethane was cooled in an ice/water bath and treated with 220 mg (1.15 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The mixture was stirred for 18 h under a calcium sulfate drying tube and was then poured into three volumes of ether. The mixture was washed sequentially with water, saturated sodium bicarbonate solution, water, 10% potassium bisulfate solution, water, and saturated sodium chloride solution. The organic layer was dried over magnesium

sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel flash chromatography, using 2:1 hexane: acetone as eluant, to yield 0.56 g of 1 as a white, foamy solid.

TLC: Rf=0.29 in 3% methanol/dichloromethane; HPLC, method A, Rt = 15.11; [^{1}H] NMR (300 MHz) consistent with structure; elemental analysis, calculated for 0.25 moles acetone solvate: C, 67.79, H, 6.80, N, 9.94. Found, C, 67.99, H, 6.80, N, 9.69.

Example 2

Synthesis of Fmoc-(tert-butyl)-threonyl-prolyl-phenylalanyl-4-nitroanilide (2)

1. Boc-prolyl-phenylalanyl-4-nitroanilide (18)

A solution of 2.85 g (10.0 mmol) of phenylalanyl-4-nitroanilide, 2.15 g (10.0 mmol) of Boc-proline and 2.79 mL (20.0 mmol) of triethylamine in 250 mL of acetonitrile was treated with 4.42 g (10.0 mmol) of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate. The mixture was stirred for 6 h under a calcium sulfate drying tube and was then concentrated in vacuo. The residue was dissolved in dichloromethane and washed sequentially with water, 10% potassium bisulfate solution, saturated sodium bicarbonate solution, and half-saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, filtered and concentrated, and the residue purified by silica gel flash chromatography, eluting with a gradient of 25%, 30%

and 50% acetone in hexane. Concentration to a small volume yielded a pale yellow precipitate which was filtered and dried in vacuo: yield 4.11 g. HPLC, method A, Rt = 14.71.

2. Prolyl-phenylalanyl-4-nitroanilide hydrochloride salt (19)

A 1.93 g (4.0 mmol) portion of 18 was treated, under nitrogen, with 10 mL of 4N HCl in dioxane. The mixture was stirred for 1 h, then concentrated in vacuo over KOH to yield 1.62 g of the title compound as a solvated whit solid, which was used without subsequent purification.

TLC: Rf = 0 in 40% acetone/hexane.

3. Fmoc-(tert-butyl)-threonyl-prolyl-phenylalanyl-4-nitroanilide (2)

A suspension of 405 mg of 19 in 12 mL of dichloromethane was treated with 0.35 mL of diisopropylethylamine and cooled in an ice/water bath under nitrogen. Fmoc-(tert-butyl)-threonine (397 mg, 1.0 mmol) was added, followed by 442 mg (1.0 mmol) of benzotriazol-1-yloxytris (dimethylamino)phosphonium hexafluorophosphate. After ca. 10 min., the solution was treated with 8 mL of acetonitrile, and the mixture was stirred for 16 h at ambient temperature. The mixture was concentrated in vacuo; the residue was taken up in dichloromethane and washed sequentially with water, 10% potassium bisulfate solution, saturated sodium bicarbonate solution, and half-saturated sodium chloride solution. The organic

layer was dried over magnesium sulfate, filtered and concentrated and the residue purified by silica gel flash chromatography, eluting with 30% acetone in hexane to yield 641 mg of 2 as an off-white solid.

TLC: Rf = 0.33 in 35% acetone/hexane; [^{1}H] NMR (300 MHz) consistent with structure; elemental analysis, calculated for 0.25 moles acetone solvate: C, 67.68 H, 6.30, N, 9.02. Found C, 67.81, H, 6.51, N. 8.79.

Example 3

Synthesis of Boc-valyl-prolyl-phenylalanyl-4-nitroanilide (3)

A portion of 19 was prepared from 241 mg (0.5 mmol) of 18 as described for the preparation of 2. The entire product of this reaction was taken up in 4 mL of dichloromethane, cooled in an ice/water bath, and treated with 110 mg (0.5 mmol) of Boc-valine, 68 mg (0.5 mmol) of 1-hydroxybenzotriazole monohydrate and 96 mg (0.5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The resulting mixture was adjusted to a pH of 6-7 by addition of diisopropylethylamine and stirred for one hour. The cold bath was removed, and stirring was continued for 16 h. The mixture was diluted with 3 volumes of ether, and washed sequentially with water, saturated sodium bicarbonate solution, 10% potassium bisulfate solution, water, and saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo to yield 268 mg of 3 as a pale yellow, foamy solid.

TLC: Rf = 0.37 in 35% acetone/hexane; [^{1}H] NMR (300 MHz) consistent with structure; elemental analysis, calculated: C, 61.95, H, 6.76, N, 912.04. Found, C, 61.62, H, 6.89, N, 11.70.

Example 4

Synthesis of Fmoc-cyclohexylalanyl-pipecolic acid benzyl ester (4)

1. (S)-Cyclohexylalanine (20)

A solution of 12.5 g (75.7 mmol) of (S)-phenylalanine in 50 mL of acetic acid and 35 mL of water was treated with 630 mg of platinum oxide. The mixture was evacuated and placed under an atmosphere of 47 psi of hydrogen. After the mixture was shaken vigorously for 2 days, the semisolid reaction mixture was treated with 80 mL each of water and acetic acid, filtered through a pad of diatomaceous earth, and the filtrate concentrated in vacuo. The residue was twice triturated with ether and filtered, and the resulting white solid dried in vacuo to yield 13.77 g of 20.

2. Fmoc-(S)-cyclohexylalanine (21)

A 1.82 g portion of 20 was suspended in 30 mL of dichloromethane, treated with 1.59 mL (12.5 mmol) of chlorotrimethylsilane, and stirred for 40 min. The mixture was treated with 30 mL of acetonitrile and an additional 1 mL of chlorotrimethylsilane. After stirring for 10 min., the resulting solution was cooled in an ice/water bath and treated sequentially with 3.92 mL

(22.5 mmol) of diisopropylethylamine and 2.72 g (10.5 mmol) of 9-fluorenylmethyl chloroformate. The mixture was stirred for 16 h under a nitrogen atmosphere, warming slowly to ambient temperature. The mixture was concentrated in vacuo and the residue partitioned between ether and 10% potassium bisulfate solution. The organic layer was washed sequentially with 10% potassium bisulfate solution and water and then treated with saturated sodium bicarbonate solution. An oily yellow third layer was formed, which was collected along with the aqueous layer. The ethereal layer was extracted twice more with aqueous sodium bicarbonate, each time collecting the aqueous and third layers. The combined aqueous and oily yellow layers were treated with 6M HCl to bring the pH to 1. The mixture was extracted three times with 3:1 ether: dichloromethane. These organic extracts were combined and washed sequentially with water and saturated sodium chloride solution, and then dried over magnesium sulfate. The mixture was filtered and concentrated in vacuo to yield 3.55 g of 21 as a pale yellow solid; [^{1}H] NMR (300 MHz) consistent with structure.

3. Fmoc-cyclohexylalanyl-pipecolic acid benzyl ester (4).

A 1.18 g (3.00 mmol) portion of 21 was converted to its acid chloride by reaction with 0.52 mL (6.0 mmol) of oxalyl chloride in 20 mL of dichloromethane in the presence of catalytic amount (3 drops) of dimethylformamide for 1.5 h followed by concentration in vacuo. A solution of this acid chloride in 10 mL of dichloromethane was treated with 691 mg (3.15 mmol) of (S)-

pipecolic acid benzyl ester 4-toluene sulfonic acid salt, followed by 10 mL of saturated sodium bicarbonate solution. The mixture was stirred vigorously for 5 minutes and the layers separated. The organic layer was dried over magnesium sulfate and concentrated in vacuo to yield a yellow foam which was purified by silica gel flash chromatography, eluting with 15% acetone in hexane to yield 566 mg of 2 as a colorless oil.

TLC: Rf = 0.19 in 15% acetone/hexane; HPLC method B, Rt = 22.81; [^{1}H] NMR (300 MHz) consistent with structure.

Example 5

Synthesis of Boc-alanyl-prolyl-leucine benzyl ester (5)

1. Boc-alanyl-proline (22)

To a slurry of 0.7 g of 5% palladium on carbon in 50 mL of ethanol was added a solution of 6.79 g (18.0 mmol) Boc-alanyl-proline benzyl ester. The mixture was placed under an atmosphere of hydrogen and stirred for 20 h, then filtered through a pad of diatomaceous earth, washing the filter pad with 20 mL of ethanol. The combined filtrates were concentrated in vacuo, dissolved in 300 mL of saturated sodium bicarbonate solution, and washed twice with 150 mL of ether. The aqueous portion was acidified to pH 2 with sodium bisulfate and extracted three times with 150 mL of ethyl acetate. These organic extracts were combined, washed with saturated sodium chloride, dried over magnesium sulfate, filtered and concentrated to yield 4.9 g of 22 as a white solid; [^{1}H]

NMR (300 MHz): d 1.35 (d, 3H), 1.43 (s, 9H), 2.07 (m, 3H), 2.35 (m, 1H), 3.59 (m, 1H), 3.74 (m, 1H), 4.48 (m, 1H), 4.81 (m, 1H), 5.35 (d, 1H).

2. Boc-alanyl-prolyl-leucine benzyl ester (5)

To a slurry of leucine benzyl ester 4-toluene sulfonic acid salt (0.40 g, 1.02 mmol) in 10 mL of dichloromethane was added, at 0°C, 0.177 mL (1.02 mmol) of diisopropylethylamine, 0.115 g (0.85 mmol) of 1-hydroxybenzotriazole hydrate, and 243 mg (0.85 mmol) of 22. The pH of the solution was adjusted to 6 with additional diisopropylethylamine and 182 mg (0.93 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride was added. The mixture was stirred for 14 h, then treated with water (75 mL) and ethyl acetate (75 mL). The aqueous layer was extracted twice with additional ethyl acetate, and the combined organic layers were washed sequentially twice with water, then with 0.5 N hydrochloric acid, saturated sodium bicarbonate solution, and saturated sodium chloride solution, then dried over magnesium sulfate and filtered. The filtrate was concentrated in vacuo and the residue was purified by silica gel flash chromatography, eluting with 25% ether in dichloromethane to yield 0.34 g of 5 as a white foamy solid.

TLC: Rf = 0.43 in 5% methanol/dichloromethane; HPLC method A, Rt = 12.75 [^{1}H] NMR (300 MHz) consistent with structure.

CLAIMS

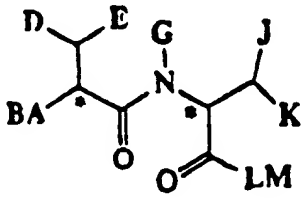
1. A linear, modified peptide, which has an affinity for FK-506 binding protein and a backbone similar in length to that of a peptide of two or three amino acids.

2. A linear, modified peptide of Claim 1, having an inhibitory effect on the peptidyl-prolyl cis-trans isomerase activity of FK-506 binding protein.

3. A linear, modified peptide of any one of Claims 1 and 2 having immunosuppressive activity.

4. A linear, modified peptide of Claim 3, having at least one hydrophobic side chain or protecting group.

5. A linear, modified peptide having immunosuppressive activity, represented by the general formula:



wherein:

a) A is NH, O, S or CH, wherein:

if A is NH, O, or S, B is PCO- or POCO-, where P is selected from the group consisting of C1-C6

straight alkyl, C1-C6 straight alkenyl, C1-C6 branched alkyl, C1-C6 branched alkenyl group, C5-C6 cycloalkyl, C5-C6 cycloalkenyl, and methyl having a substituent which is independently selected from the group consisting of C5-C6 cylcoalkyl, C5-C6 cyclo-alkenyl, phenyl, 1-naphthyl, 2-naphthyl, 9-fluorenyl, and 1-adamantyl; or

if A is CH, then B is connected via a trans double bond and is selected from the group consisting of C2-C4 straight alkyl, C2-C4 straight alkenyl, C2-C4 branched alkyl, C2-C4 branched alkenyl, and methyl or ethyl substituted with either a C5-C6 cyclic alkyl group or Ar, where Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, phenyl and phenyl having one to three substituents which are independently selected from the group consisting of hydroxyl, halo, nitro, CF_3, C1-C4 straight alkyl, C1-C4 branched alkyl, C1-C4 straight alkenyl, C1-C4 branched alkenyl, O-(C1-C4) straight alkyl, O-(C1-C4) branched alkyl, O-(C1-C4) straight alkenyl, O-(C1-C4) branched alkenyl, and Ar, as defined above,
wherein no more than two Ar groups may be linked together;

b) D is selected from the group consisting of hydrogen, C1-C4 straight alkyl, C1-C4 branched alkyl, C1-C4 straight alkenyl, C1-C4 branched alkenyl, hydroxy, tert-butyloxy, benzyloxy, 4-benzyloxyphenyl, cyclohexyl, $-(CH_2)_n-CO_2-Q$, where

n is 0 or 1 and Q is methyl, ethyl, i-propyl, t-butyl, benzyl, 1-naphthyl, 2-naphthyl, or cyclohexyl, and Ar as defined above,
wherein no more than two Ar groups may be linked together;

c) E and K are independently hydrogen or methyl;

d) G is either methyl or ethyl;

e) J is selected from the group consisting of hydrogen, C1-C6 straight alkyl, C1-C6 branched alkyl, C1-C6 straight alkenyl, C1-C6 branched alkenyl, C5-C6 cycloalkyl, C5-C6 cycloalkenyl, sulfhydryl, hydroxy, phenyl, 3-indolyl, and benzyl, wherein J may be connected to G by a bond to form a cycle of 5 or 6 members; and

f) L is 0 or is an α-amino acid residue attached via the α-nitrogen, and selected from the group consisting of alanine, 2-aminobutyric acid, valine, norvaline, leucine, norleucine, isoleucine, phenylalanine, cyclohexylalanine, tryptophane, 1-naphthylalanine, 2-naphthylalanine, threonine with a benzyl ether side chain, threonine with a tert-butyl ether side chain, glutamic acid with a tert butyl ester side chain, glutamic acid with a benzyl ester side chain, methionine, serine with a benzyl ether side chain, and serine with a tert-butyl ether side chain, wherein:

if L is O, then M is selected from the group consisting of C1-C6 straight alkyl, C1-C6 branched alkyl, C1-C6 straight alkenyl, C1-C6 branched alkenyl, and $-(CH_2)_n$-Ar, where n is 1-6 and Ar is as described above,
wherein no more than two Ar groups may be linked together; or

if L is an amino acid, then M is selected from the group consisting of O-(C1-C4) straight alkyl, O-(C1-C4) branched alkyl, O-benzyl, NH-phenyl, and NH-4-nitrophenyl and is attached to the amino acid carbonyl.

6. A linear modified peptide of Claim 5, wherein B, D or M is a bulky hydrophobic moiety.

7. An immunosuppressive linear, modified peptide of any one of Claims 5 and 6, having an affinity for FK-506 binding protein.

8. A linear, modified peptide of Claim 5 having an inhibitory effect on the peptidyl-prolyl cis-trans isomerase activity of FK-506 binding protein, where said compound is selected from the group consisting of Fmoc-alanyl-pipecolylphenylalanyl-4-nitroanilide, Fmoc-(tert-butyl)- threonyl-prolyl-phenylalanyl-4-nitroanilide, Boc-valyl-prolyl-phenylalanyl-4-nitroanilide, Fmoc-cyclohexylalanyl-pipecolic acid benzyl ester, Boc-alanyl-prolyl-leucine benzyl ester, Fmoc-(tert-butyl)-glutamyl-prolyl-phenylalanyl-4-nitroanilide, Boc-alanyl-prolyl-alanine benzyl ester, Boc-alanyl-prolylphenylalanine benzyl ester, Boc-alanyl-prolyl(tert-butyl)glutamic acid methyl ester, Boc-alanyl-prolyl-phenylalanine methyl ester Boc-(benzyl)-tyrosyl-proline benzyl ester, Boc-(benzyl)-tyrosyl-proline tert-butyl ester, and Fmoc-alanyl-pipecolic acid benzyl ester.

9. A composition for suppressing an immune response in an individual, comprising an immunosuppressive linear, modified peptide, which has an affinity for FK-506 binding protein, and a backbone similar in length to that of a peptide of two or three amino acids, and a physiologically acceptable vehicle.

10. A composition for suppressing an immune response in an individual comprising an immunosuppressive linear, modified peptide of Claim 5 having an affinity for FK-506 binding protein and a physiologically acceptable vehicle.

11. A composition for preventing or reducing graft rejection associated with a bone marrow or organ transplantation in an individual, comprising an immunosuppressive linear, modified peptide of Claim 5 and having an affinity for FK-506 binding protein, and a physiologically acceptable vehicle.

12. A composition for preventing or reducing an autoimmune response in an individual comprising an immunosuppressive linear, modified peptide of Claim 5 and having an affinity for FK-506 binding protein, and a physiologically acceptable vehicle.

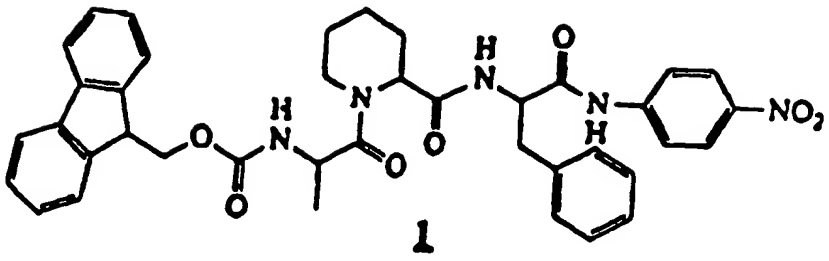
13. The composition of any one of Claims 9-12, having an inhibitory effect on the peptidyl-prolyl cis-trans isomerase activity of FK-506 binding protein, wherein the immunosuppressive peptide is selected from the group consisting of Fmoc-alanyl-pipecolyl-phenylalanyl-4-nitroanilide, Fmoc-(tert-butyl)-threonyl-prolyl-phenylalanyl-4-nitroanilide, Boc-valyl-prolyl-phenylalanyl-4-nitroanilide, Fmoc-cyclohexylalanyl-pipecolic acid benzyl ester, Boc-alanyl-prolyl-leucine benzyl ester, Fmoc-(tert-butyl)-glutamyl-prolyl-phenylalanyl-4-nitroanilide, Boc-alanyl-prolyl-alanine benzyl ester, Boc-alanyl-prolylphenylalanine benzyl ester, Boc-alanyl-prolyl-(tert-butyl)glutamic acid methyl ester, Boc-alanyl-prolyl-phenylalanine methyl ester, Boc-(benzyl)tyrosyl-proline benzyl ester, Boc-(benzyl)tyrosyl-proline tert-butyl ester, and Fmoc-alanyl-pipecolic acid benzyl ester.

14. The composition of any one of Claims 9-12, further comprising administering the composition with an immunosuppressant selected from the group consisting of CsA, rapamycin, FK-506, 15-deoxyspergualin, OKT3, and azathioprine.

15. A composition of any one of Claims 9-12, further comprising administering the composition with a steroid.

FIGURE 1

a)



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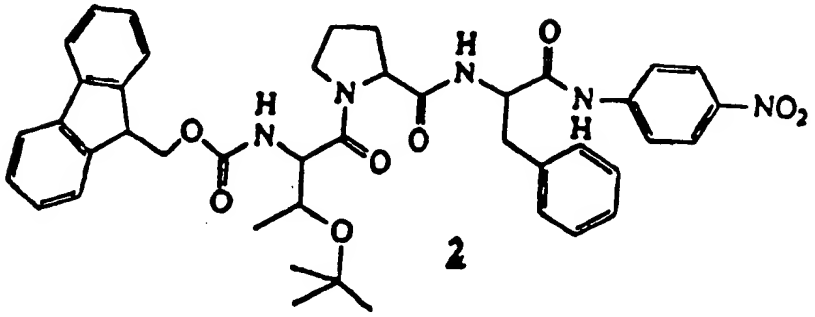
b)



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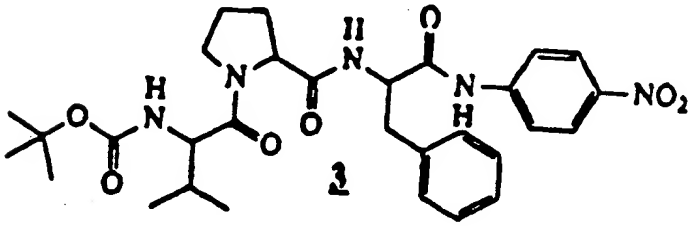
c)



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d)

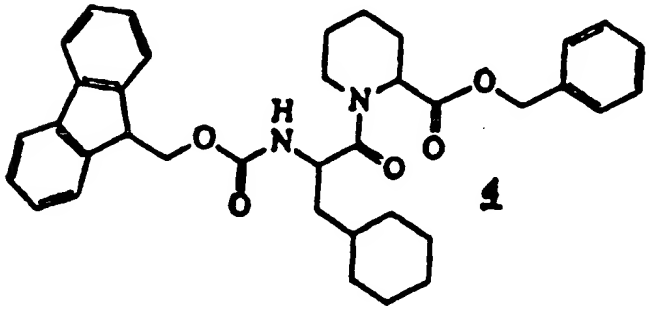

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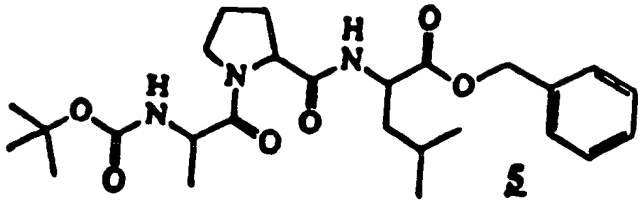
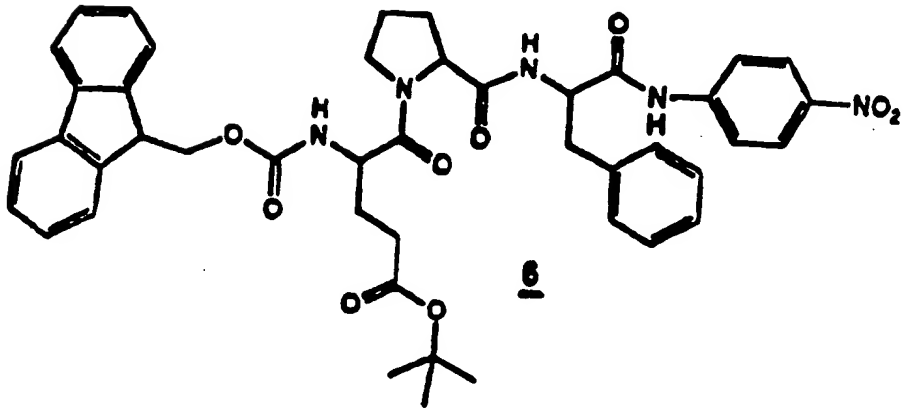
FIGURE 1 (Cont.)

e)



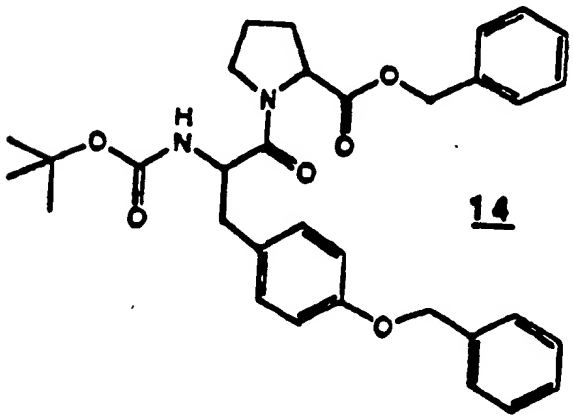
5

f)



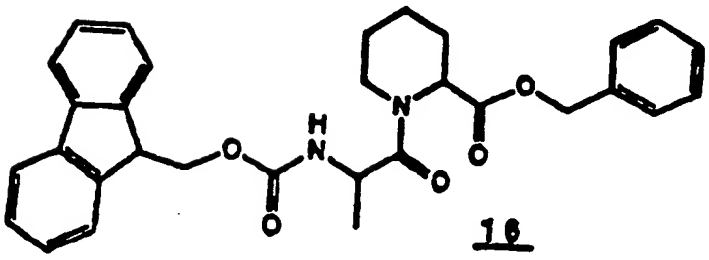
6

g)



14

h)



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INTERNATIONAL SEARCH REPORT

PCT/US 91/05691

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K5/06; C07K5/08; A61K37/64

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[9]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	BIOCHEMISTRY. vol. 24, 1985, EASTON, PA US pages 2040 - 2047; TANAKA, T ET AL: 'Human Leukocyte Cathepsin G. Subsite Mapping with 4-Nitroanilides, Chemical Modification, and Effect of Possible Cofactors' * The whole document, especially p.2046, col 2, l.16-17 * --- -/--	1-13

[9] Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 JANUARY 1992	24 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	KORSNER S.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 110, 1989, Columbus, Ohio, US; abstract no. 18543D, MISCHAK, R.P. ET AL: 'Administration of immunotoxins and immunosuppressants for the inhibition of immune responses during tumor treatment' page 61 ;column 2 ; see abstract & WO,A,8 806 451 (XOMA CORP.) 7 September 1988 ---	14

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9105691 SA 52213

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 10/01/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-8806451	07-09-88	AU-A- 1421488	26-09-88
		EP-A- 0303681	22-02-89
		JP-T- 1502909	05-10-89